PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patent Office
Canberra

I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003901871 for a patent by VISION BIOSYSTEMS LIMITED as filed on 31 March 2003.

COMMONWEALTH OF AUSTRALIA
AUSTRALIA
PATENT OFFICE

WITNESS my hand this
Second day of July 2003

J. Billingsley

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

VISION BIOSYSTEMS LIMITED

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A method and apparatus for fluid dispensation, preparation and dilution"

The invention is described in the following statement:

A METHOD AND APPARATUS FOR FLUID DISPENSATION, PREPARATION AND DILUTION

An apparatus and method will be described for dispensing and preparing fluids. In one form the apparatus and method relate to dispensing and prepared fluids on samples by an automated biological reaction apparatus.

Fluids used in reactions on samples may require dilution or mixing prior to application or reaction taking place. One particular use of fluids is the dilution or mixing of reagents applied to tissue samples by an automated biological reaction apparatus, such as that described in Australian Provisional patent application No PS 3114 titled "A method and apparatus for providing a reaction chamber", filed 20 June 2002. The embodiments described herein relate to such a device, but also may have applicability to a number of apparatus for applying reagent to samples on slides, particularly apparatus designed to automate reagent application to slides.

Specific examples of methods and apparatus for fluid dispensation and preparation will be discussed, with reference to the following figures:

Figure 1 shows a mixing station located on an instrument used to apply fluids to samples;

Figure 2 shows the mixing station of Figure 1;

Figure 3 shows an insert for the mixing station of figure 2;

An example of an apparatus 10 used to apply fluids, such as reagent, to samples, is shown in figure 1. Apparatus 10 is a biological reaction apparatus as described in Australian Provisional Patent Application No. PS 3114 titled Automated Biological Reaction Instrument filed on 20 June 2002 by the present applicant. The contents of the aforementioned document are hereby incorporated by reference.

The apparatus 10 includes a robot arm 16 having a pipette 28 connected to pumps (not shown) by tubing 29. The apparatus has a number of bulk reagent containers 20, slide trays 15, and a reagent rack receptacle 36 for receiving reagent racks. A single reagent rack 34, as shown in figure 2, may support a number of reagent containers 39.

In the present example, the apparatus 10 may be loaded with one or more slide trays 15, three of which are shown in figure 1. Each slide tray 15 will have at least one slide (not

shown), and each slide typically contains a tissue sample (not shown). The slide may also contain a slide identifier, such as a bar code, which uniquely identifies the slide and the sample contained thereon. In the present embodiment, the samples on the slides are covered by a covertile (not shown), which protects the sample from dehydration and provides a reaction chamber for reagents, which are applied by the pipette 28 of the apparatus 10.

When a slide tray 15 is loaded, the robotic arm 16 moves to be adjacent the slide tray 15, and a bar code reader (not shown) mounted to the robotic arm 16 reads the bar code on each slide. The information relating to the slide is then stored by a controller (not shown) for the apparatus 10 so that the sequence and type of reagent to be applied to the sample may be determined. The controller, for example, may be a stand-alone computer connected to the apparatus 10 over a network, or may be an on-board controller.

There are usually a limited number of reagent containers 39 that may be accessed by the apparatus 10. In the apparatus 10, there are four reagent trays 34, each holding a maximum of nine reagent containers 39, for a maximum of thirty six reagent containers 39. Each reagent container may be independent of the other reagent containers, and each reagent container includes a unique bar code identifier (not shown). When a reagent rack 34 containing a number of reagent containers 39 is loaded onto the apparatus 10, the robotic arm 16 moves along the reagent rack 34 to scan the bar codes on each reagent container 39. Information relating to reagent content and position of individual reagent containers is stored in the controller of the apparatus.

Other reagent containers such as bulk reagent containers 20, are included in the body 12 of the apparatus 10, adding to the type of reagents that may be dispensed onto the slide. Some bulk reagent containers 20 normally contain fluids required for washing and hydrating samples.

The reagent rack 34 may be used to contain a detection kit. A detection kit consists of a number of reagents in separate reagent containers 39 that are used to perform a particular test on one or more samples. Such a detection kit may include nine reagent containers 39 to perform a single test, and this reduces the number of reagent containers 39 available to other slides to twenty seven.

Typical reagents applied to samples on slides include primary antibodies, such as those sold by Novocastra Laboratories Ltd. These reagents are normally supplied in the reagent containers 39 in volumes typically between 7ml and 30ml. Other reagents and fluids, such as buffers and de-ionised water, may be kept in the bulk storage containers 20 which typically have volumes between 1-4 litres.

Some reagents, once prepared for application to a sample, have a relatively short shelf life. Therefore, either the reagent is supplied pre-mixed in a ready-to-use formulation, whereupon it must be used within a short period of time from ordering, or it may be prepared by laboratory staff prior to use, and placed into an appropriate reagent container. Some of the reagents, such as 3', 3 – diamino benzidene (DAB), when in a final form, begin to degrade soon after preparing and may not be useable more than 24 hours after initial preparation. This requires a new batch to be prepared every day, and ensuring that old batches are discarded after use. Further, enzymes such as protease may need to be applied in varying concentrations depending on factors such as tissue type, other reagents to be applied etc. This can result in numerous batches of reagents being required to be prepared before application to the samples, with the associated problems such as correct application, expiry date, correct mixing, tracking and traceability.

Concentrated primary antibodies may also require preparation before use, requiring dilution before application to a sample. Primary antibodies can be supplied either in a concentrated form or pre-diluted ready-to-use. However, it may be necessary to have several different working dilutions of the same antibody on a single apparatus 10, which would otherwise take up several locations in the reagent rack 34. It is therefore advantageous to have a single reagent container 39 of an antibody, where diluting of the antibody reagent may take place before the reagent is applied to the sample. The primary antibody may be diluted by a primary antibody diluent such as ABDIL 9352 sold by Vision BioSystems Ltd.

In the present embodiment of the apparatus 10, a mixing station 122 is provided, as shown in figure 2. Mixing station 122 includes an insert 130 having a number of mixing vials 132 as shown in figure 3. The insert 130 has six vials, each vial able to hold a different reagent. The vials 132 are shown all the same volume, but may vary in volume according to requirements. Typical volumes may be 7 ml per vial.

Also mounted to the insert 130 is a tab 134. Tab 134 may be used to identify the insert 130 such as by way of a barcode. It is envisaged that as the insert 130 is disposable, but may contain a number of different reagents over the course of several runs of the apparatus 10.

The bar code on the insert 130 may be used to identify the insert 130 so that the controller knows when to discard the insert 130, and request that a new insert be loaded into the mixing station 122. This may be predetermined after a set period of time or uses.

Also shown on insert 130 is an overflow aperture 135, which is adapted to allow excess fluid to drain from the insert should any of the vials 132 overflow.

In use, information from the slide bar codes may be cross-checked with a database in the controller to establish which series of reagents is to be applied to each slide. The apparatus 10 then compares the reagents required, to the reagents currently loaded. If a reagent is identified that is not in final form for application to a sample, then a preparing step is scheduled into the order of tasks to be undertaken on the apparatus 10.

In one example, three reagent containers (identical to reagent container 39 located in the reagent rack 34) each have a component part A, B, and C of DAB may be located on the apparatus 10. In the present example DAB will be mixed in a ratio of 1 part A to 25 part B to 1 part C. To mix a batch of DAB ready for use, the robotic arm 16 first moves to the reagent container containing part A, and withdraws a set volume of part A of the reagent. The robotic arm 16 then moves to one of the vials 132 at the mixing station 122 and deposits the volume into one of the vials 132. The pipette 28 then moves to a washing station located next to the mixing station 122, where the outside and inside of the pipette 28 are rinsed. Once cleaned, the robotic arm 16 moves the pipette 28 to the reagent container containing part B of the reagent. The pipette 28 withdraws the reagent (25 times the volume of part A) and moves to the vial containing part A. Once deposited in the vial, the pipette 28 moves to the washing station and is again washed, before moving to the reagent container holding part C of the reagent. The same volume as removed from the container holding part A is removed, and the pipette 28 moves to the original vial and deposits the reagent with the other reagents. Initially depositing the reagents into the mixing vials causes some mixing, however additional mixing can be accomplished by withdrawing some or all of the reagent from the vial 132 into the pipette 28, then re-

depositing the reagent into the vial 132. The pipette may move vertically to ensure that the tip is above the fluid level when depositing to aid the mixing process. The energy of re-deposition causes the reagents to mix more readily. This mixing process can be undertaken a number of times as desired. After the reagent has been mixed sufficiently, the pipette 28 may proceed to the wash station if the next reagent to be applied to a sample is not DAB. This volume of the vials and the amount withdrawn by the pipette provide a sufficient volume of DAB for many applications to samples. Whenever DAB is required, the robotic arm moves the pipette 28 to the vial where the DAB was mixed, as the vial in which mixing of particular reagents is recorded by the controller. The time of the preparation is also recorded, so that after a predetermined period of time the mixed reagent can be discarded. This prevents the prepared reagent from being used after expiring.

After completion of testing for the day, or at the expiry of the DAB, the vial 132 containing the DAB (or any other reagent that has expired) can be cleaned as discussed below.

In relation to scheduling of mixing within a batch, specific details of scheduling are disclosed in Australian Provisional Patent application titled "Method of Scheduling" filed 24 February 2003 by same applicant, the contents of which are hereby incorporated by reference.

While the above process is automated, the resources employed (robotic arm 16 and pipette 28) may be utilised for significant periods of time in general reagent application to samples, and therefore it may be desirable to reduce the necessity to prepare several batches of reagent during a day. For this reason the apparatus 10 can be programmed to prepare reagents in the absence of any samples loaded into the apparatus 10 or during normal processing, and the volume and concentrations are user determinable through a user interface (not shown).

In the above example the concentration and time of preparation of each reagent in each vial 132 are stored in the memory of the controller of the apparatus 10, so there is no chance of old or incorrect mixed reagent being applied to a sample, reducing operator error.

The mixing by the pipette 28 ensures that the prepared reagent is fully mixed before application to a sample, and provides a better uniformity of mixing than, for example, applying components of the reagent directly to the sample and mixing on the sample.

Other examples of reagents that benefit from mixing on the apparatus 10 include protease, which may be required to be applied in a number of concentrations. In the above example, only one reagent container of protease would be required, and several concentrations of protease may be prepared by the apparatus 10 using diluent stored on board either in a reagent container 39 or bulk reagent container 20. These different concentrations may be placed in different vials 132 for later use.

In the above example, it is possible to have the mixing tasks scheduled into the steps of applying reagent to the samples. For example, there are often periods of time during a testing of a slide where there are no tasks required of the robot arm. These times may be referred to as open times, which typically occur when the fluid applied to a slide requires time to react before the next step is undertaken. If an open time is of a sufficient length, it may be possible to schedule in a mixing step. This minimises the time required to complete the application of fluid to samples, while freeing the operator from preparing the reagents.

After reagent is prepared, and it is applied to samples, remaining or expired prepared reagent is siphoned to waste by the aspirator. The vials 132 may then be cleaned. Cleaning is undertaken by draining any prepared reagent remaining after the required prepared reagent has been dispensed. Draining is done with the pipette 28, the drained fluid being directed to an internally plumbed bulk waste container. Once substantially empty, a rinse cycle is undertaken. The rinse cycle may use a cleaning solution, which for example could contain an alcohol such as IMS dispensed into the vial 132. The cleaning solution is then drained via the pipette 28. More than one rinse cycle may be undertaken. After removing cleaning solution for the final rinse, any remaining cleaning solution is allowed to evaporate to completely empty the vial.

It is also possible to revisit the mixing vial after a predetermined time from initial preparation, to re-mix the reagent. This may be done by withdrawing some of the prepared reagent into the pipette 28, and redispensing into the same vial 132. This may be

important where components of the prepared reagent settle after time or do not stay mixed after a period of time. As with initial mixing, the remixing step may be scheduled during a period of inactivity of the robot arm and aspirator.

DATED this 31st day of March, 2003

VISION BIOSYSTEMS LIMITED

By DAVIES COLLISON CAVE
Patent Attorneys for the applicant

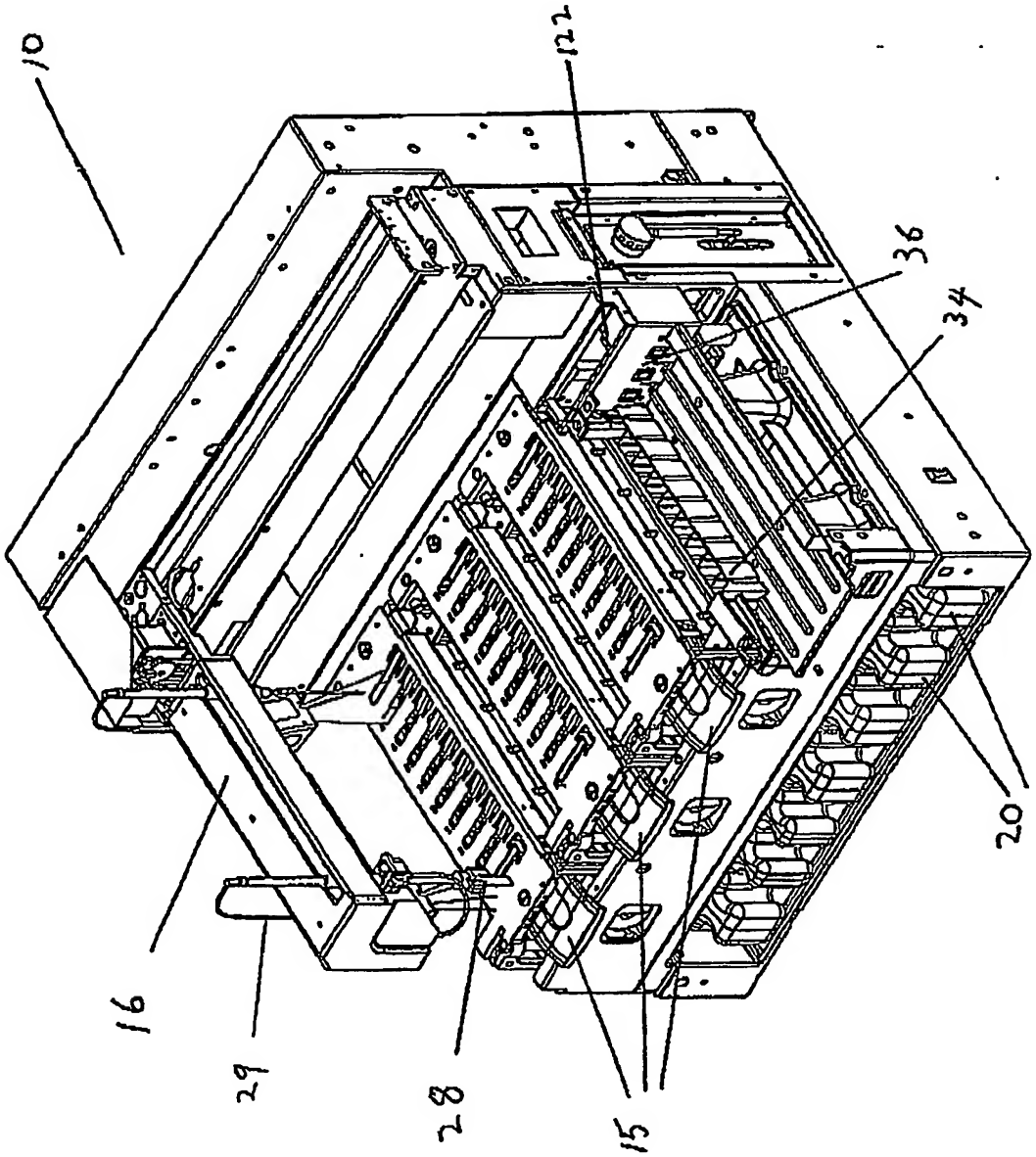

Figure 1

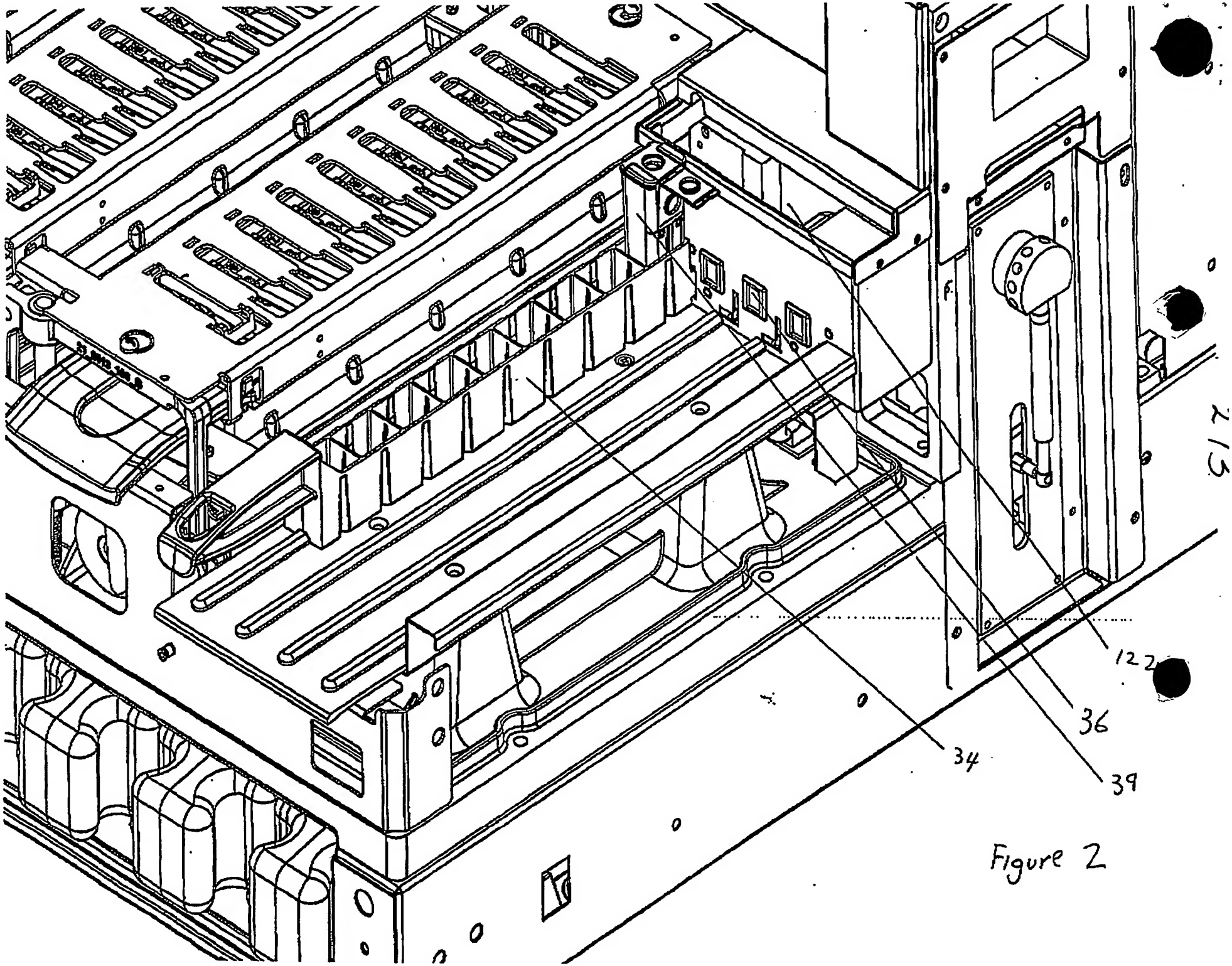

Figure 2

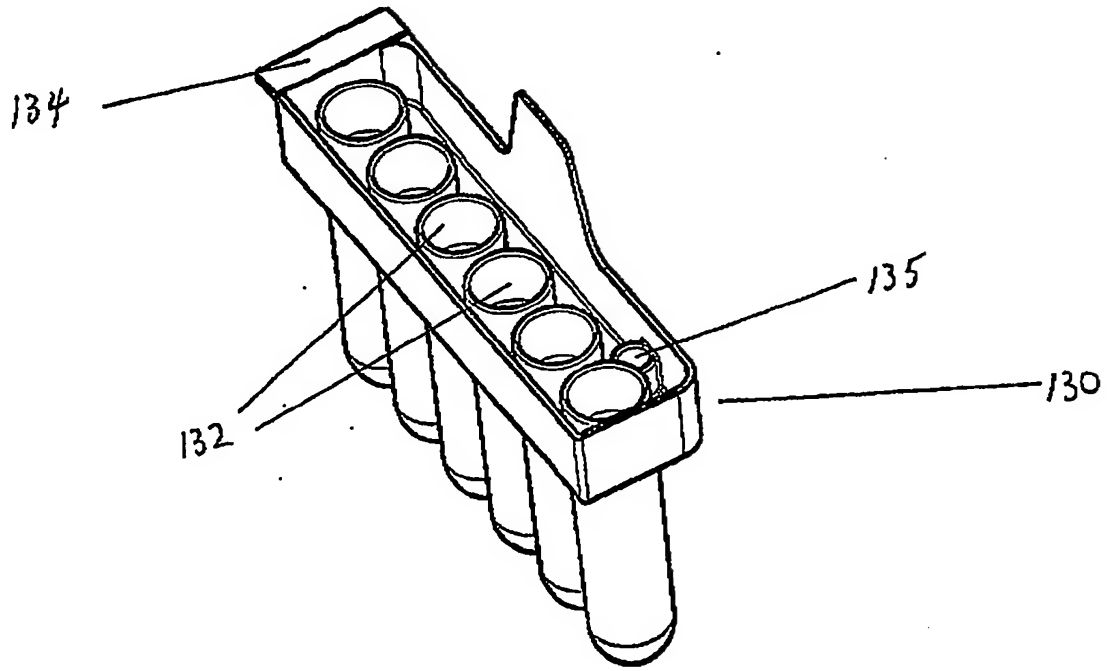

Figure 3